

On the iodine test for semen / by Wyatt Johnston.

Contributors

Johnston, Wyatt, 1863-1902.
Royal College of Surgeons of England

Publication/Creation

[Boston] : [publisher not identified], [1897]

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p. c. 9

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Reprinted from the Boston Medical and Surgical Journal of April 8, 1897.

ON THE IODINE TEST FOR SEMEN.¹

(Glycerin reaction)

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If one were asked to instance a couple of medico-legal tests generally conceded to be sufficiently reliable to be accepted without hesitation as a positive proof of the condition they were presumed to indicate, one would not be unlikely to select as examples the hemin test in the case of blood stains and the recognition of spermatozoa in stains due to semen as fulfilling these requirements, when properly performed by competent persons.

While this is no doubt true, it has long been recognized that the technique at our disposal for the examination of spots supposed to consist of semen leaves very much to be desired.

It is by no means certain that all the stains sworn to from time to time as being seminal have really been such, so much does the testimony concerning these objects depend on the degree to which caution and experience have tempered the personal skill and acuteness of the expert. The identification of spermatozoa with absolute certainty, while easy enough in a fresh stain, becomes increasingly difficult with the lapse of time; and the incidental handling, washing or wearing of the articles of clothing, which commonly require this examination, makes it more and more problematical whether the spermatozoa can be demonstrated entire and intact.

There are numerous extraneous objects which are so like the detached heads and tails of spermatozoa as to mislead even those who are thoroughly experienced in the work. The generally accepted rule is that no body which simply resembles the head or tail of a spermatozoon should be considered as serious proof, and the search must be continued until perfectly formed and entire spermatozoa are recognized. The uncertainty produced by finding substances resembling these heads and tails in the specimens examined, may, however, lead the expert to prolong needlessly the examination of stains which are not seminal at all. Delays from this cause may have serious results in judicial procedure, simply by retarding or preventing the public exoneration of innocent persons wrongly suspected. We have also, on the other hand, the possible miscarriage of justice owing to the experts or juries attaching importance to incomplete proof, where the other circumstances of the case are such as to arouse strong suspicions, and must not lose sight of the consequences of abandoning a search for spermatozoa because they could not be promptly found.

¹ Read before the Society, October 3, 1896.

Thus, in addition to a possibility which cannot be altogether ignored that the bodies affirmed to be spermatozoa were not so in reality, we have the serious drawback that the expert can only feel perfectly certain that the stain is non-seminal after making protracted and laborious observations; these, if omitted, may leave a crime unpunished in the case of a genuine stain not received in good condition.

The difficulties mentioned do not imply by any means that the spermatozoa test is not a good one, but simply that it is not adequate for the task it has had to fulfil of being the sole and only recognized test for semen. What is evidently wanted is some simple, prompt, preliminary test, by which one could decide in a few minutes whether a given stain is likely to repay a search microscopically for spermatozoa, or not. At the same time such a test would evidently be still more useful if it was sufficiently accurate and distinctive to afford good corroborative evidence of the presence of semen, and so to guard against the possibility of the expert being led to regard as spermatozoa some entirely extraneous substances.

A test which appears in great part to fill those conditions has recently been announced by A. Florence, of Lyons, whose monograph entitled "*Du Sperme et des Taches de Sperme en Médecine Legale*,"² contains much besides that is of interest.³

Florence recommends the use of a reagent not infrequently employed for testing alkaloids, known as the ter-iodide or tri-iodide, of potassium. This reagent is a solution of iodine and potassium iodide, in proportions which correspond to the formula KI_3 but which is not a definite chemical combination. The formula recommended is as follows:

Iodide of potassium C. P.	1.65 grammes
Iodine	2.54 grammes
Distilled water	30 c. c.

Good results can also be obtained by one-half the amount of iodine (1.27 grammes equivalent to KI_2), but the iodine must be in excess. The well-known Gram's solution is not suitable for the reaction. The solution keeps perfectly well in glass-stoppered bottles. It should be used cold, as warmth interferes with the reaction.

When a drop of the liquid obtained by moistening a seminal stain is placed alongside a drop of the above solution on a glass slide or watchglass, so that the edges of the drops come in contact, there appear almost immediately large numbers of peculiar, brownish-red, pointed crystals. These are rhomboidal, and resemble so closely in form, size and color the hemin crystals that they could readily be mistaken for them, though a careful examination shows points of difference. This comparison to hemin crystals will convey a better idea of their appearance than could be given by the most elaborate description, and will enable them to be distinguished from the crystals which are met with in seminal fluids. They also form small turnstile groups or crosses, in the manner which characterizes the hemin crystals.

² Archives d'Anthropologie Criminelle, January, February and March, 1896.

³ It has also been published separately by Storek, Lyons.

According to Florence (and my own observations confirm his statement), the crystals are sparingly soluble in cold water and very soluble in warm water, reappearing again on cooling. On exposure to the air, they gradually disappear, but reappear on adding a fresh quantity of the reagent. They are readily soluble in ether, alcohol, acids, fixed alkalies and iodide of potassium; they resist solution in very weak ammonia solutions. For their formation to be typical and abundant considerable dilution is necessary, and I have found the degree of this solution a most important detail in making the test.

Dried stains usually give the reaction in a manner fully as prompt and typical as fresh semen, and I have been using as a demonstration specimen for class use a stain on cotton over twelve months old, obtained from a homicide case. Recently, the crystals have been more difficult to obtain in abundance, the reason being apparently the increased difficulty of obtaining a solution of the semen rather than an impairment of the reaction.

The exact nature of the crystals has not been shown, as far as I am aware; I have regarded them as some special crystalline form of iodine.

The nature of the substance which occasions the reaction has not yet been thoroughly established. Florence claims to have isolated from seminal stains a substance which he terms *viriespermine*, and which he regards as a distinctive body producing this phenomenon. He states that it does not correspond in reactions with the substance isolated and called *spermine* by Poehl. It is said to be very soluble and to resist completely the effects of ammoniacal decomposition.

By using a larger quantity of material, the reaction can be obtained in the test-tube, an abundant red or chocolate-brown deposit of crystals being thrown down. In this way the demonstration of the seminal character of a stain might even be made without the use of a microscope.

A single fibre teased out of a thread in a piece of cotton stained by semen is sufficient to give a profuse crop of crystals under the microscope.

As to the degree to which this reaction is characteristic of semen: Florence claims that it will not react with any of the other secretions of the body, such as blood, urine, sweat, saliva, tears, bile or milk, nor with pus or nasal or vaginal mucus. The secretion of the Cowper's glands does not give it. I have made a number of tests with the various substances mentioned above, and have always obtained negative results, or at all events have never obtained a characteristic reaction.

The sperm of animals is stated by Florence not to give the reaction, as far as his observations went, though these were not extensive. Personally, I have not tested this point with sufficient thoroughness to give an opinion; but as far as my observations go a pseudo-reaction of doubtful nature can be obtained with at least some forms of animal semen. This point is still, as far as I know, unsettled.

In respect to Florence's claim to priority, I think it can hardly be disputed. I can find no recent work on legal medicine in which any chemical test is given for semen. Wood⁴ says, "There are no chemical tests by which seminal stains

⁴ Vol.ii of Witthaus and Becker's Handbook, 1894, p. 79.

can be recognized." Dixon Mann (1893), Strassman (1895), Vibert (1896, 4th Edition), Hoffman, Taylor, Liman and our other standard authors do not refer to it. Roussin indeed recommended for examining seminal stains the use of solution of iodine and iodide of potassium, but only for the purpose of staining the spermatozoa, and the fluid which he recommended (iodine 1, iodide of potassium 4, water 100) does not give the Florence reaction. Apart from Florence's work there is practically no literature on the subject except the older work of Orfila as to the odors obtained upon heating the stains or treating them with nitric acid.

Florence states that he started with the firm conviction that so unusual a fluid as semen, which had such well-marked physical peculiarities, must contain some characteristic chemical substance. Acting with this hypothesis in view he proceeded *seriatim* to test seminal stains with all the ordinary reagents used in obtaining chemical reactions, especially those found of value for recognizing alkaloids and those generally employed in physiological chemistry. By trying these one after the other he discovered several which gave him positive results, and among these he selected the ter-iodide of potassium as the one best adapted for medico-legal requirements.

During the past three months I have been making some observations on cadavers (22 cases) upon the occurrence of this reaction in connection with the secretions from the prostate, seminal vesicles, testicle substance and the post-mortem ejaculations from the meatus, with a view of determining whether the prostatic ingredient of the semen or the semen proper was chiefly concerned in giving the reaction. The material was obtained for the most part by allowing it to dry on cotton-wool swabs, so as to obtain a condition comparable with those under which seminal stains ordinarily come under medico-legal examination.

Pressure of other work has prevented me making these examinations with sufficient thoroughness to make their publication in detail seem advisable until I have gone over the material again more carefully, but the general results are as follows: Drying does not appear to interfere with the reaction materially within the time limits I have mentioned; and, in fact, I have often obtained the reaction more satisfactorily from moistening the dried secretion than from the original fluid. Decomposition, such as is met with in drowned bodies and bodies long exposed to the air, appeared to interfere with it to some extent, contrary to what Florence's observations would lead us to expect. The semen from the meatus or from seminal stains gave a better reaction than that substance obtained from the regions where the prostatic and testicular components of the semen had not yet mingled. Semen expressed from the prostatic duct into the urethra gave prompt and characteristic results, while these were much harder to obtain from the testes or the contents of the vesicles. So much was this the case that at first I thought the reaction might be due to the prostatic element of the secretion and not to the strictly seminal part. In some cases, however, typical results were obtained from the contents of the seminal vesicles and from the substance of the testicles. In two cases the reaction was imper-

fect or almost absent, certainly not sufficiently typical to justify medico-legal deductions, one being a case of cancer of the seminal vesicles and prostate, and the other double chronic vesiculitis with corpora amylacea very abundant in the prostate. In both of these, spermatozoa were present in the semen.

Hypertrophy of the prostate did not appear to affect the reaction. In one case the reaction appeared to be present before the period of puberty (seven and one-half years), though here it did not appear to be perfectly typical and satisfactory. I could not find it in the secretions of infants and very young children.

These few observations are not recorded here so much with a view of passing judgment upon the percentage reliability of the test as to indicate the direction in which observations might be made, as the total number of observations I have been able to make so far is not sufficient to justify general conclusions.⁵

With regard to the practical merits of the test, I think that those who use it for testing seminal stains will agree as to its decided value as a preliminary test, and accord it a position analogous to the guiacum test for the blood, by which we can promptly find out whether there is a probability of positive results being obtained upon further examination. Personally, if it was obtained in a typical manner, I should regard it as very strong corroborative evidence in a case where spermatozoa were claimed to be present.

With regard to its independent value as a test, Florence formulates his conclusions as follows:

(1) From the occurrence of the reaction alone, without discovering even fragments of spermatozoa, he would conclude that it was probably a seminal stain.

(2) From the coincidence of well-formed and typical but detached heads of the spermatozoa, along with the reaction, he would affirm positively the presence of semen.

(3) With *débris* of spermatozoa or even with perfectly-formed heads, but without the reaction, he would not feel certain that he was dealing with a stain produced by human semen.

It will be noticed that Florence attaches to the detached head of spermatozoa more significance than is accorded to them by our standard authorities. It must be mentioned that Florence has added materially to the data which may be used in identifying the heads of human spermatozoa, as the result of his careful study by means of oil immersion lenses and staining methods. By these more refined methods, highly characteristic details were brought out which would escape notice if ordinary dry lenses of moderate magnification were the only ones employed. The illustrations of spermatozoa given in his monograph are far superior to the illustrations which adorn the average medico-legal text-book, which are in many cases little more than caricatures. It must be remembered that the drying and subsequent moistening of the spermatozoa have a tendency to deform them somewhat, so that the appearance

⁵ Two instances of advanced and well-marked disease of the seminal receptacles is obviously a very high proportion for only 22 cases. In both these two instances of organic disease the fact of iodine reaction being interfered with while the spermatozoa were still present indicates the greater certainty of the spermatozoon test.

obtained may not always be perfectly uniform. This tendency to deviation would, however, be more likely to cause human semen to be confounded with that of animals, than the reverse. Personally, my experience does not entitle me to express an opinion as to the extent to which detached heads and tails of spermatozoa should be allowed to constitute evidence of semen, but I think it will be a long time before anything less than the entire spermatozoon will be accepted as legal tender by either judges or juries.

I think it can be said with confidence that the iodine reaction of semen, as described by Florence, is a decided step in advance, and that in it we have a new and very promising preliminary and confirmatory test for normal semen, and one which gives relatively good results in the case of stains which from a prolonged drying, react with difficulty to the microscopic test.

The sources of fallacy which attend the iodine test are, however, as yet practically unknown.

To determine what inference may be drawn from the absence of this reaction in a suspected stain, we need much fuller information and experiments on the effects of external conditions, etc., upon substances known to be stained by semen; we also need information as to the extent to which pathological conditions may exert a modifying influence, how the age limit affects the reaction, and on many other points.

Before we can assign accurately the significance of a positive result, we require much further testing and corroboration regarding the behavior of the reagent with substances other than semen, and also with the semen of animals.

After making all due allowance for this we must rejoice at the success of M. Florence in discovering a crystalline reaction for semen. As a rule, a good crystalline is better than a color reaction and we may expect that further researches in this direction will lead to the discovery of new and valuable micro-chemical tests.



